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Anfal Nuri AbbasAllaftah and Amal Ali Yaseen AlHassan



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Lavender Oil Anti-Oxidative and Anti-Tumor Activity

Anfal Nuri AbbasAllaftah^{a)}, Amal Ali Yaseen AlHassan^{b)}

Biology Department, College of Education for Pure Sciences Basra University, Iraq.

^{a)} Corresponding author: anfal.abbas@uobasrah.edu.iq

^{b)} amal.yaseen@uobasrah.edu.iq

Abstracts. "Abstract" Antioxidant and Anti-tumor efficacy of commercial lavender oil has been studied on Human Hepatocyte cancer cell line HCAM with eight concentration (0,5, 6, 7, 8, 10,12 & 20) $\mu\text{g/ml}$. Cell viability was measured after 48hrs. Result show the Rate of growth viability of HCAM cell line treated with eight concentration of Lavender oil $\text{IC}_{50} = 7.916 \mu\text{g/ml}$. Acridine / ethidium bromide is used to identify changes in cancer cell membranes during programmed cell death. That showed stages of programmed death of Tumor cells : as the oil extract destroys plasma membrane thus its death completely . antioxidant activity of lavender oil showed the high value of oil extract as antioxidant it's no reactive oxygen species (ROS) content.

INTRODUCTION

Lavandula (common name lavender) One of the genus of the plant family Lamiaceae whose members are distinguished by the essential and aromatic oils that have proven their efficiency in the pharmaceutical and cosmetic fields, ; those members are also known as ornamental plants. [1;2;3] Plants are a natural gift and day after day Is their effectiveness is proved in combating many diseases that kill humanity. Many plants and their effective compounds Have been tested for their ability to inhibit and curb Tumor cells . [4;5;6]

Numerous studies have shown that phytotherapy is promising hope to overcome many of the side effects known techniques that deal with Tumor cells. [7,8].

For each stage of Tumor development in human body; initiation, promotion and progression require special treatment and different chemotherapy approaches. Chemotherapy is specific to a Promotion stage for most types of cancer ; As the role revolves around Reducing proliferation of tumor cells , induction of tumor cell differentiation and increased rate of cell death[9].

Since natural products have played an important role in drug research, active plant compounds have been a major contributor to the production of the medicinal drug, and several studies have as anti-cancer agents. [10,11,12] Essential oils (EOs) have been shown to suppress cancer cells but not non-cancerous cells and tissues.

Research is still invested in Finding modern methods and techniques that are optimal for cancer treatment, such as loading active compounds on nanoparticles and studying the efficiency of plant materials in distinguishing receptors on cancer cell walls [12, 13, 14].

MATERIALS AND METHODS

Lavender Essential Oils

Lavender oil was imported from the Australian company pure black. <https://www.purelyblack.com.au/en/>



Cell Line

The HCAM cancer cell line was obtained from the IRAQ Biotech Cell Bank Unit in Basrah and maintained in RPMI-1640 supplemented with 10% fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were reseeded at 50 % confluence twice a week using Trypsin-EDTA and incubated at 37 °C and 5 % Co2[17].

Cytotoxicity Assay

To determine the cytotoxic effect, the MTT cell viability assay was conducted on 96-well plates. Cell line HCAM were seeded at 1×10^4 cells/well. After 24h or a confluent monolayer was achieved, cells were treated with the tested Lavender oil with six concentration (5, 6, 7, 8, 10 & 12) µg/ml. Cell viability was measured after 48hrs. Adding 28 µL of 2 mg/mL of MTT solution by removing the medium (and incubating the cells for 2 h at 37 °C. After removing the MTT solution, the remaining crystals in the wells were dissolve by adding 100 µL of DMSO (Dimethyl Sulphoxide) accompanied by incubation of 37 °C for 15 min with shaking[17]

The absorbency on a microplate reader was measured at 620 nm (test wavelength); assay equation:

$$\text{Proliferation rate as (PR)} = B/A * 100$$

A is the mean optical density of untreated wells

B is the optical density of treated wells and IR= 100- PR [18]

Detection of Cell Death by Staining With Acredine Orange & Ethedium Bromide (AO&EB)

Cultured cells were stained with Acredine Orange & Ethedium Bromide (AO/EB) after 72 hours of treatment, examined with fluorescence microscopy and digital camera imaging, the live cells were stained green and the dead cells were stained red. [19]

Antioxidant activity determination

Antioxidant activity of the lavender oil determine by use reactive oxygen species (ROS) fluorometric assay kit produce by Elabsciece (United States compouny). <https://www.elabscience.com/>

RESULTS

Cytotoxicity Lavender Oil Invitro

The concept of the half minimum inhibitory concentration is used in the world of pharmacological research as an indicator of the effectiveness of inhibition of a biological or biochemical substance. The

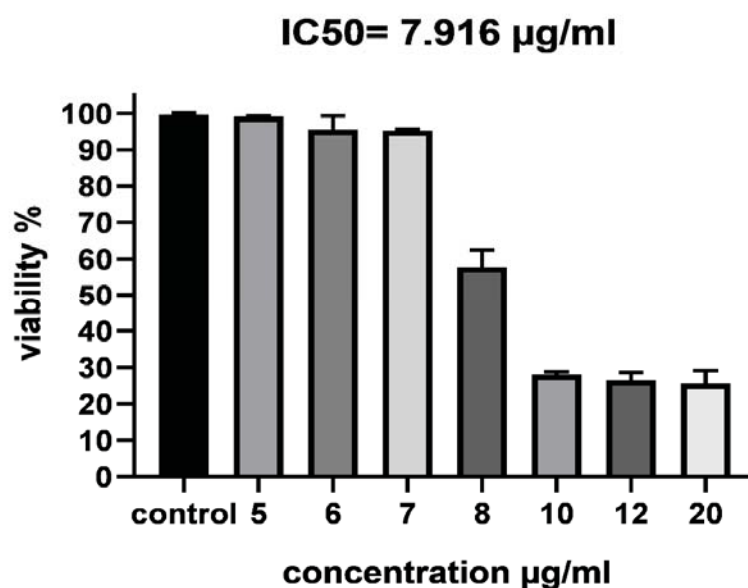
value of IC_{50} indicates for the minimum inhibitory concentration required to inhibit a specific biological substance or vital function by the half; The lower value the more effective substance [20]

Lavender Oil Cytotoxicity Was determined Invitro by using MTT assay,

HCAM cell line treated in different Concentrations of lavender oil

Results showed that Rate of growth viability of HCAM cell line treated with eight concentration of Lavender oil $IC_{50}= 7.916 \mu\text{g/ml}$. As shown in the table and diagram

Con. $\mu\text{g/ml}$	5	6	7	8	10	12	20
Mean Viability%	99.1	95.5	95.2	57.8	28.	26.5	25.6



Detection of Cell Death by Staining with Acridine Orange & Ethidium Bromide (AO&EB)

Acridine / ethidium bromide is used to identify changes in Tumor cell membranes during programmed cell death [21], as it can be accurately distinguished between the different stages of programmed cell death. [22], Cancerous hepatocytes were treated with half inhibitory concentration of lavender oil extract. Acridine Orange/Ethidium Bromide (AO/EB) Staining used to Detect Apoptosis

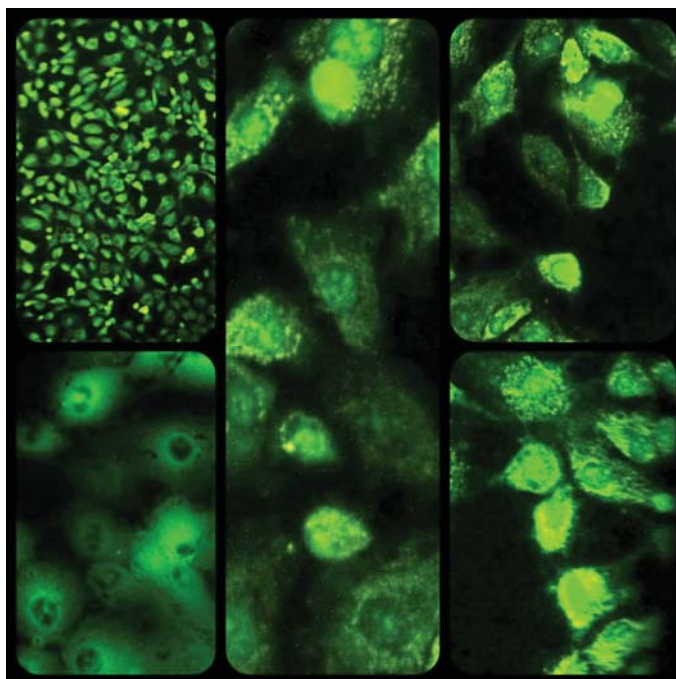


FIGURE 1. Control–HCAM cancer cell line without treatment.

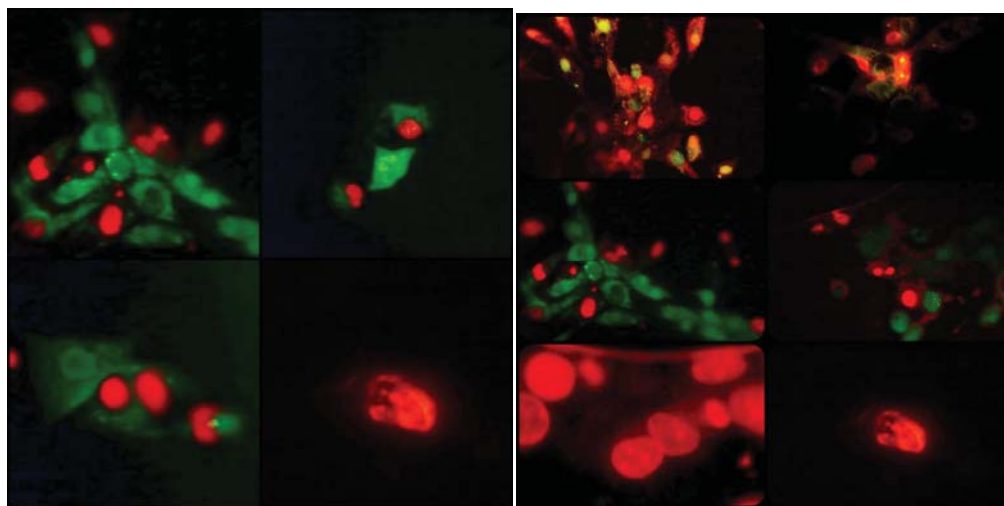


FIGURE 2. HCAM cell after treatment with lavender oil and that appear how they started killing apoptotic cells in red refer to dead cells and cells in green to live cells and cells that appear in two colors (green and red together) are liver cells that suffer from programmed death due to the effect of oil.

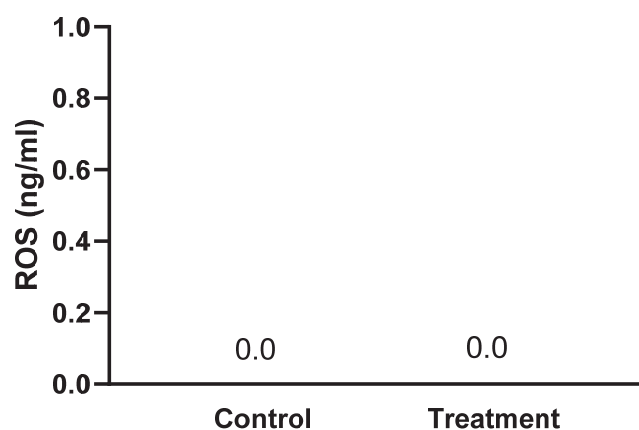
Determination of Antioxidant Activity

Lavender oil extract has a very high effectiveness as an antioxidant due to its containing polar compounds that are able to give the proton to scavenge free radicals and thus it can be used to inhibit the growth of cancerous tumors; as the oil extract of lavender was able to scavenge all free radicals by donating the protons.

Recent studies have shown that the extract owning a value of half the inhibitory concentration less than 50µg/ml were high in antioxidant [23, 24] antioxidant activity of lavender oil showed the Important role of oil extract as antioxidant with no reactive oxygen species (ROS) content results of reactive oxygen species (ROS) are shown in the Table. There are many unsaturated fatty acids in plant oils, which are most sensitive to oxidation degradation. Plant oils are also widely used as a substrate for oxidative activity evaluation. [25.26]

TABLE 2. The antioxidant activity of lavender oil

Sample	ROS concentration	Unit	Note
1	0.0	ng/ml	Control 1
2	0.0	ng/ml	Control 2
3	0.0	ng/ml	Treatment 1
4	0.0	ng/ml	Treatment 2



DISCUSSION

The oil extracted from lavender is considered safe oil by studying the effectiveness of cytotoxicity in various studies [27] crude lavender oil was used in the current study Without separating its Active substances ; so the anti-carcinogenic activity was due to all the chemical components involved in the oil. It may explain the reason why essential oils are so effective in tumor cells to cannabinoid (CB) receptors in selected human cancer types This matter requires cytological and diagnostic studies to find out how the lavender oils extractor works on these cellular receptors. Many studies have suggested that cannabinoids and endocannabinoids inhibit tumor growth directly in vitro and in animal tumor models through a variety of mechanisms. Several forms of cancers are inhibited in their development and progression. Several studies have examined the chemical analysis of the components of lavender oil and were able to determine their classifications.

Each plant chemical group has its own method of identifying cancer cells through receptors on the surfaces of these cells and working to create new compounds commensurate with the type of receptors.[28,29] The word phytochemical protection against cancer is described as "the use of most substances from plant assets such as vitamins or plant proteins to control cancer cells, whether by preventing their transformation into cancer cells ; inhibiting them and sometimes delaying their development and preventing their growth and spread.[30,31] The results of the current study agreed with a number of studies that praised the role and efficiency of lavender oil in eliminating cancer cells of all kinds. The role of lavandulyl acetate as an anti-cancer and anti-tumor has been demonstrated by the results of many studies. [32,33,34,35,36] The most important mechanics in eliminating cancer cells that essential oils target is the event of programmed cell death (Apoptosis) or the act of stopping or delaying cell division.[37]

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